Ontogeny of the Catalytic Subunit and Putative Glucose-6-Phosphate Transporter Proteins of the Rat Microsomal Liver Glucose-6-Phosphatase System

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The catalytic subunit (p36) and putative glucose-6-phosphate (G6P) transporter (p46) protein levels of the rat glucose-6-phosphatase (G6Pase) system were studied in relation to G6Pase hydrolytic activity and G6P uptake in liver microsomes during the fetal to neonatal period. The mean G6P hydrolytic activity in liver microsomes increased significantly from the 20th to 21st day of gestation (from 6 to 22 mU/mg protein) and was further enhanced by 3-fold 6 hours after birth, with a maximal activity at 1 day of age (112 mU/mg protein). In contrast, G6P uptake into the vesicles was undetectable before birth, appeared after day 1 (656 pmol/mg protein), and decreased after day 2 (about 330 pmol/mg protein). Immunoblot analysis showed that the mean p36 protein level was low (<1.6 arbitrary units [AU]) during gestation, increased sharply (to about 4.0 AU) during the first day, and remained stable afterward. Unlike p36, p46 protein was present before birth at values comparable to those postpartum. P46 increased from 3.2 AU at 20 days to 4.6 AU at 21 days of gestation, and decreased transiently after birth. These results show that (1) G6Pase hydrolytic activity before birth can occur without detectable G6P uptake function; (2) the presence of the putative G6P transporter protein is not sufficient to elicit G6P uptake; and (3) full G6Pase activity requires optimal expression of both p36 and p46 proteins. These data are discussed in relation to the function of G6Pase.
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GLUCOSE-6-PHOSPHATASE ([G6Pase] EC 3.1.3.9) catalyzes the hydrolysis of glucose-6-phosphate (G6P) to phosphate and glucose and is therefore an obligatory step for glucose production by gluconeogenic tissues such as liver and kidney. The important role of G6Pase in the control of blood glucose homeostasis is exemplified in the neonatal period. In mammals, liver gluconeogenesis is markedly stimulated after birth, due to the high glucagon to insulin ratio resulting from the dietary changes associated with suckling.[1] This property is essential for maintaining glucose homeostasis during the first week of life and has been established in several animal species.[2] In breast-fed babies, galactose[3] and other gluconeogenic substrates maintain active gluconeogenesis, but appropriate liver glucose production depends on the maturation of the G6Pase system. Impaired hepatic G6Pase activity has indeed been suggested to cause fatal hypoglycemia in infants who are at risk of sudden infant death syndrome.[4,5] At birth, a burst in the activity of liver G6Pase is well documented[6-9] and corresponds to the brisk increase in the mRNA abundance of the 36-kd catalytic subunit (p36) of the G6Pase system.[8-10] Liver p36 is indeed induced by cyclic adenosine monophosphate and repressed by insulin.[9,11,12] In contrast, the mRNA level of the 46-kd putative G6P transporter (p46)[13] is already detectable in murine liver at the 17th day of gestation, increases to maximal levels until birth, and declines to adult levels after birth.[14]

In the present study, we show that the developmental profile of both the p36 and p46 proteins parallels their previously reported gene expression levels. Our results also demonstrate that G6Pase hydrolytic activity and G6P uptake functions are dissociated during development as previously indicated by our group[15] and others,[8,16] and this is reflected at the protein level by the fact that although it is already present during gestation, p46 may not be fully functional or mature.

MATERIALS AND METHODS

Animals

Fetal and newborn Wistar rats were bred in the Centre National de la Recherche Scientifique (Unité Propre de Recherche [UPR] 1524) laboratory (Meudon, France) as described previously.[9]

Preparation of Rat Liver Microsomes

Microsomes were prepared from rat livers sampled at 20 and 21 days of gestation, at 6 hours, and at 1, 2, and 7 days after birth. For each age, 3 individual pools from the liver of several animals were homogenized, microsomes were prepared and resuspended at a protein concentration of about 7 mg/mL in 50 mmol/L Tris hydrochloride, pH 7.3, and 250 mmol/L sucrose, and the intactness of the vesicles was measured as previously described.[17]

G6Pase Assay

G6Pase hydrolytic activity was measured as described before in untreated (intact) microsomes.[15] Briefly, the production of [U-^{14}C]glucose from 5 mmol/L [U-^{14}C]G6P was measured for 5 minutes at 30°C in 50 mmol/L Tris hydrochloride buffer at pH 7.3. The reaction was stopped by the addition of ice-cold $ZnSO_4$ (0.15 mol/L) and saturated $Ba(OH)_2$, which traps the unreacted [U-^{14}C]G6P in the Zn/Ba precipitate. The [U-^{14}C]glucose produced is recovered in the supernatant following a centrifugation at 16,000 × *g* for 1 minute and the radioactivity is counted in a scintillation counter. One unit of G6Pase activity corresponds to the amount of enzyme that produces 1 µmol glucose/min in the conditions of the assay.

G6P Uptake

G6P uptake was measured as described previously in detail in untreated (intact) microsomes.[18] The accumulation of [U-^{14}C]–

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associated molecules (mainly radioactive glucose[18]) in microsomes was measured in an incubation medium identical to the one used for the determination of G6Pase activity, but with 0.2 mmol/L [U-^{14}C]G6P and at room temperature (23°C) for 1 minute (at which time steady-state accumulation was reached), followed by filtration in a Millipore (Bedford, MA) apparatus equipped with cellulose nitrate filters. The filters were further washed 3 times and the radioactivity on the filters was counted in a scintillation counter. [U-^{14}C]G6P uptake was defined as the amount of [U-^{14}C]-labeled molecules accumulated in the microsomes in 1 minute, and is expressed as picomoles per milligram of microsomal protein.

Antibodies, Western Blot Analysis, and Enzyme-Linked Immunosorbent Assay

A synthetic peptide (5-GYGYYRTVIFSAMFGGY-21) corresponding to the NH_2-terminal end of p46 was covalently linked to a protein carrier (KLH and bovine serum albumin) as described in the Imject immunogen EDC conjugation kit from Pierce (catalog no. 77101; Pierce, Rockford, IL). New Zealand rabbits were immunized by subcutaneous multisite injections first with complete Freund adjuvant and then with incomplete Freund adjuvant from Sigma (St Louis, MO). Boosting doses were administered at 0, 14, 28, 42, and 56 days with 250 to 500 µg peptide each time. Immune and preimmune sera were tested as described previously[19] against specific and nonspecific peptides by enzyme-linked immunosorbent assay (ELISA) and against microsomal preparations by Western blotting. The ELISA results were quantified with a Dynatech (Chantilly, VA) MR 7000 at 405 nm. The rabbit polyclonal antiserum against the recombinant G6Pase catalytic subunit (antibody against p36) was a kind gift from Dr J.Y. Chou (National Institutes of Health, Bethesda, MD). Microsomal fractions (50 µg protein) were subjected to 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis at 100 V for about 1 hour in Laemmli buffer. Proteins were then electroblotted onto a nitrocellulose membrane at 100 V for 1 hour. The membrane was saturated for 1 hour in 100 mmol/L TBS, pH 7.5, containing 10% dried milk (wt/vol) and further incubated overnight at 4°C in the primary antibody solution (diluted to 1:500 for anti-p36 or 1:200 for anti-p46). After washing, the membrane was incubated in the alkaline phosphatase–conjugated anti-rabbit IgG solution (diluted to 1:2,000) for 2 hours, washed again, and detected in the BCIP/NBT substrate system (Promega, Madison, WI). The membrane was scanned and the quantification of each specific band was analyzed using a Dual Light Transilluminator, and these data are expressed as arbitrary units (AU) corresponding to the intensity of the revealed band divided by its area.

RESULTS AND DISCUSSION

Western Blot Analysis of Developmental Changes in p36 and p46 G6Pase Proteins

Immunodetection and the relative abundance of the p36 protein in microsomes were assessed with a polyclonal antibody raised against the catalytic subunit of G6Pase. The immunoreactive band migrated to a M_r of about 36 kd (Fig 1A), and the corresponding density scan results are shown in Fig 1B. The p36 immunoreactivity was low (about 1.5 AU) during the last days (21 to 22) of gestation, as well as 6 hours after birth, and sharply increased at 1 day postpartum (by >2-fold).

The putative G6P transporter component of the G6Pase system of about 46 kd, p46, was detected by an antibody raised against its *N*-terminal part (Fig 2A). The scan results of the specific band are shown in Fig 2B. Unlike p36, p46 was already present in 20-day-old fetal rats at levels (3.2 ± 0.5 AU) similar to those found in 7-day-old rat liver microsomes (3.3 ± 0.4 AU) and transiently increased to 4.6 ± 0.03 AU at 21 days of gestation ($P < .05$, n = 3).

There was thus a very different developmental profile for the p36 catalytic subunit and the p46 putative G6P transporter protein of the G6Pase system, suggesting that G6P transport capacity preceded G6Pase activity. We therefore investigated the relationship of these profiles with respect to G6Pase activity and G6P uptake in microsomes.

Developmental Changes in Liver Microsomal G6Pase Hydrolytic Activity and G6P Uptake

Figure 3A shows that G6Pase activity was low (6.3 ± 0.9 mU/mg protein) in liver microsomes isolated from 20-day-old fetal rats, but increased steadily until 6 hours after birth by about 10-fold. Lower G6Pase activities versus postnatal values were also found in human fetal liver.[20] The activity was maximal (112 ± 17 mU/mg protein) at 1 day postpartum and declined by about 10% 1 day later. Surprisingly, G6P uptake was undetectable before birth and up to 6 hours after birth (Fig 3B). This function briskly appeared at 1 day postpartum (656 ± 194 pmol/mg protein) and then stabilized to lower values (329 ± 69 pmol/mg protein) at 7 days postpartum. These results demonstrate that G6Pase activity and G6P uptake are dissociated during development as first indicated by our group[15] and confirmed by others.[8,16] One explanation given was that during gestation, the active site of p36 faces the cytoplasm and therefore would not require G6P transport for activity.[16] A detailed topological analysis may confirm this intriguing possibility. In this respect, it is interesting to recall that we previously

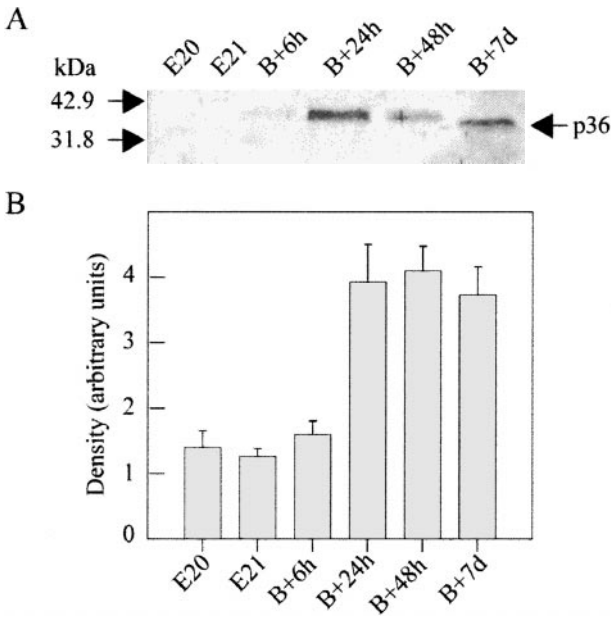

Fig 1. Western blot analysis of developmental changes in the p36 catalytic subunit protein of G6Pase. (A) One representative visualization of specific bands of p36 at different ages during development. (B) Quantification of specific bands for each age group. Results are the mean ± SEM for each group (n = 3). All values before and after 6 hours postpartum are statistically different by $P < .05$ (unpaired Student's *t* test). E20 and E21, rat embryos of 20 and 21 days of age, respectively; B + 6h, B + 24h, B + 48h, and B + 7d, newborn rats at 6, 24, and 48 hours and at 7 days of age, respectively.

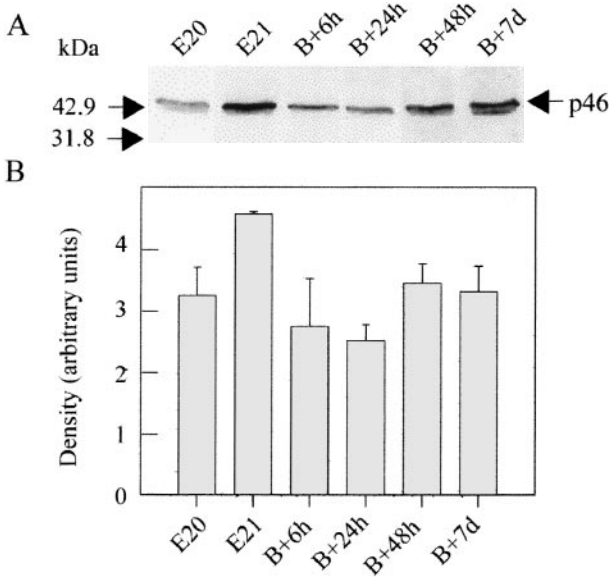

Fig 2. Western blot analysis of developmental changes in the p46 putative G6P transporter protein of G6Pase. (A) One representative visualization of specific bands of p46 at different ages during development. (B) Quantification of specific bands for each age group. Results are the mean ± SEM for each group (n = 3). Values at 21 days of gestation and 24 hours postpartum are statistically different *v* all other values (except 6 hours postpartum) by $P < .05$ (unpaired Student's *t* test).

found that only 10% of the glucose produced from G6P transits through the lumen of adult fasted rat liver microsomes,[18] demonstrating that the majority of glucose produced by G6P hydrolysis is formed outside of these microsomes. Whether this partition is modified in conditions of increased hepatic glucose production is not known, but topology studies have established that the active site of mature G6Pase catalytic subunit p36 is oriented toward the lumen of the microsomes,[21] and therefore, one may presume that conformational changes in the p36 protein may allow exposure of the active site partly to the cytosol as well. Examples of proteins with more than one topological orientation in the endoplasmic reticulum have been documented.[22]

Figure 4 clearly shows the dissociation between G6Pase activity and G6P uptake. G6Pase activity gradually increased in liver microsomes from fetal rats (E20 and E21) and neonatal rats (B + 6h) without detectable G6P uptake. However, G6P uptake briskly appeared after 6 hours postpartum while G6Pase activity further increased during this period.

Our data are consistent with the developmental profile of both p36 and p46 (GSD-1b) mRNAs[8,14,16] and have several implications. They show that (1) G6Pase hydrolytic activity before birth can occur without detectable G6P uptake function (Figs 3 and 4); (2) the presence of the putative G6P transporter protein (Fig 2) is not sufficient to elicit G6P uptake; and (3) full G6Pase activity requires the optimal expression of both p36 and p46 proteins.

We documented previously that both p36 and p46 mRNAs and proteins were regulated in parallel by insulin and cyclic adenosine monophosphate,[12] but this is not the case here. Therefore, factors other than the insulin to glucagon ratio

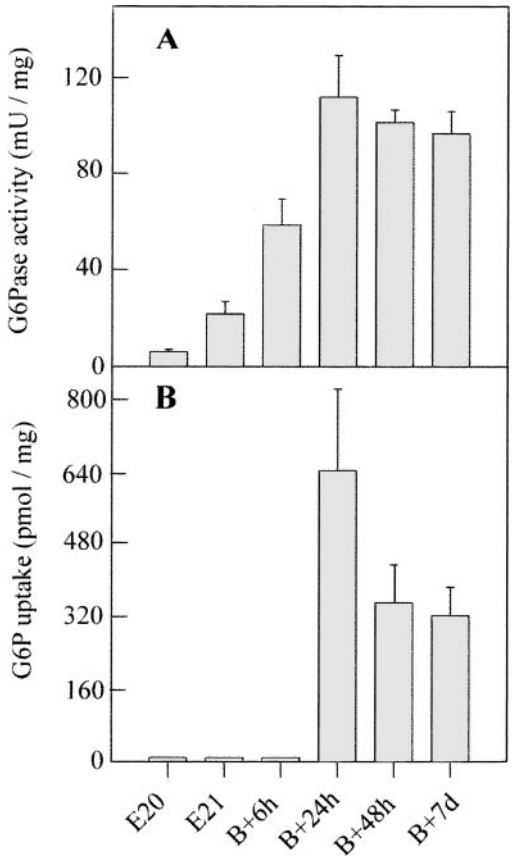

Fig 3. Developmental profile of G6Pase activity and G6P uptake in liver microsomes. (A) G6Pase activity was measured in intact liver microsomes of rats with 5 mmol/L G6P as substrate at the indicated ages. (B) [U-^{14}C]G6P uptake was measured with 0.2 mmol/L G6P in intact liver microsomes of rats at the indicated ages. G6P uptake was not detectable at E20, E21, and B + 6h.

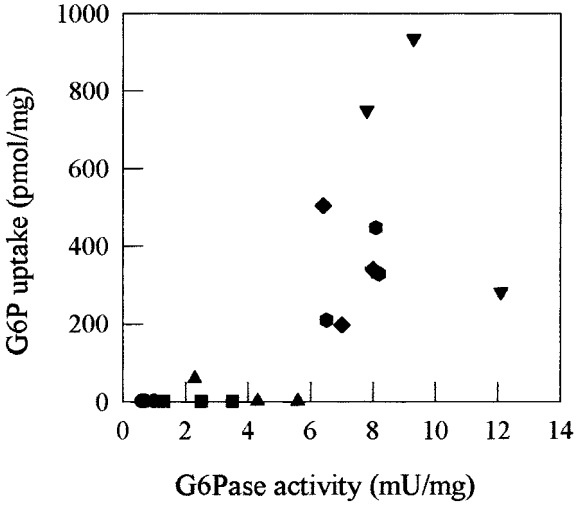

Fig 4. Correlation between G6Pase activity and G6P uptake in liver microsomes. G6Pase activity and [U-^{14}C]G6P uptake were measured as described in Fig 3 on pools of liver microsomes from fetal (●, E20; and ■, E21) and postpartum (▲, B + 6h; ▼, B + 24h; ◆, B + 48h; and ●, B + 7d) rats, respectively.

should be responsible for the different developmental profiles of p36 and p46 protein. The fact that a significant protein level of p46 is already present as early as E20 in gestation raises the question as to whether the p46 protein plays a different role during development besides its attributed G6P uptake function once it is coexpressed with p36. Hiraiwa et al[23] have postulated that p46 may have a dual role depending on which tissues or cells it is expressed in. It is tempting to hypothesize that the p46 protein might function as a ''G6P sensor'' that could regulate glycolysis during development and especially during dietary changes associated with suckling. It remains to be understood how p46 controls p36 activity and what the role of G6P uptake (if any) has in the operation of the G6Pase system in the perinatal period.

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